DOP-PCR Labeling (SKY)

Section of Cancer Genomics, Genetics Branch, NCI National Institutes of Health

Reagents

Agarose, Ultrapure
Gibco BRL, Cat. 15510-027
Ampli Taq polymerase 5 U/µl
Perkin Elmer, Part No. N808-0158
Buffer, 10X and 25 mM MgCl ₂
Perkin Elmer, Part No. N808-00
Loading buffer, 5X
Quality Biological, Cat. 51-026-030
Ethidium Bromide
Research Genetics, Cat.750007
Primer "UN1"
Midland Certified Reagent Co. Telenius 6MW
[5'-CCGACTCGAGNNNNNNATGTGG-3']
10X TAE buffer
Advanced Biotechnologies, Cat. 08-514-001
Template DNA
Use $400 - 600$ ng for each reaction
100 mM dNTP nucleotides
dGTP
Boehringer Mannheim, Cat. 1051466
dCTP
Boehringer Mannheim, Cat. 1051458
dATP
Boehringer Mannheim, Cat. 1051440
dTTP
Boehringer Mannheim, Cat. 1051482
x – dUTP:
Spectrum Green
Vysis, Cat. 30-803200
Spectrum orange (1 mM)
Vysis, Cat. 30-803000
Texas red (1 mM) Molecular Probes, Cat. C-7631
Biotin (50 mM)
Boehringer Mannheim, Cat. 1093070
Digoxygenin (50 mM)
Boehringer Mannheim, Cat. 1558706
Water, sterile (H ₂ O)
vaci, scinc (1120)

Molecular grade sterile distilled water

Materials and Equipment

PCR Thermocycler MJ – Research, Inc., Model PTC - 100 Gel system and power source PCR tubes

PGC Scientifics, Cat. 502-075

Preparation

1X TAE buffer

Dilute 10X TAE with dH₂O

1% agarose gel

Dilute 1 g of agarose into 100 ml of 1X TAE buffer, heat until dissolved

Stock dNTP 2mM

	<u>µl</u>	mM final
dGTP	10	0.2
dCTP	10	0.2
dATP	10	0.2
dTTP	7.5	0.15
dH_20	<u>462.5</u>	-
Total	500	

x - dUTP's

Spectrum Orange (SpO) is diluted 1:5 in sterile H_2O (14 μ l SpO + 56 μ l H_2O).

Texas Red (TR) is diluted 1:2 in sterile H_2O (30 μ l TR + 30 μ l H_2O). Spectrum Green (SG) is diluted 1:5 in sterile H_2O (13 μ l SG + 52 μ l H_2O)

Microcentrifuge tubes, pipette tips, and pipettes

Autoclave PCR microcentrifuge tubes (0.5 ml size), 2 ml microcentrifuge tubes, and pipette tips (10, 200, and 1000 µl).

Sterilize pipettes (using UV) to be used for PCR and only use that set for PCR (can use Stratalinker or UV light source from TC hood).

Workspace

Before starting make sure that the work space is cleaned with ethanol and there is a steady air flow; if not, then use the tissue culture hood to maintain sterility.

Template DNA

400 - 600 ng (see note 1)

Procedure

Use sterile techniques throughout, especially when handling the DNA samples.

- 1. Label PCR tubes according to labeling scheme (note 2) (e.g. 1A, 1E, etc.) plus a zero control for each color (master mix with no DNA sample).
- 2. Defrost template DNA at 37° C, vortex and spin briefly prior to aliquoting out the DNA. Aliquot 4 μ l of the appropriate DNA into each PCR tube, seal lid, and temporarily place at 4° C.
- 3. Label five 2 ml microcentrifuge tubes (A E) and make the master mix for each fluorochrome according to the table (table 1) below and put on ice. The order for pipetting the master mix is as follows: dH₂O, buffer, MgCl₂, dNTP, and primer.
- 4. Dilute the TR, SpO, and SG dUTP's immediately before use. Vortex the labeled fluor dUTP's, spin down briefly and add each to the appropriate master mix and place back on ice.
- 5. Mix the Taq enzyme carefully (tap with finger), spin down, then add appropriate amounts to each master mix. Vortex the master mix tubes, spin and put back on ice.
- 6. Put PCR tubes with aliquoted DNA in fluorochrome order (all A's in one row, all B's in the next, etc.)
- 7. Carefully open each tube in row A by handling only the outside of the tube. Vortex the master mix for SpO, pipet 96 µl into each tube (change tips between each tube) and put on ice. Repeat the same procedure for each remaining x-dUTP.
- 8. Vortex each tube, spin down briefly and put them on ice again.
- 9. Start the PCR machine and select the PCR program (note 3), arrange the tubes in the PCR machine, close lid, and start the program.
- 10. After completion, remove the tubes from the PCR machine, vortex, spin down, and place on ice. In a round bottom Elisa plate, place 0.8 μl of 5X

DNA loading buffer in each well to which you will be adding a 2 μ l aliquot from each reaction to run on a 1% agarose gel. The resulting smear migrates to around 500 bp (note 4).

Table 1.

	Spec Orange	Texas Red	Bio (Cy5)	SG	DIG (Cy5.5)
	A	В	C	D	${f E}$
10X PCR	130	120	140	150	130
buffer					
$MgCl_2$	104	96	112	120	104
(25 mM)					
1-dNT (2	65	60	70	75	65
mm)					
sterile dH20	819	756	910	945	845
Primer	52	48	56	60	52
(100 uM)					
PE	13	12	14	15	13
AmpliTaq					
polymerase					
x-dUTP	65	60	42	75	39
	(dil.1:5)	(dil.1:5)			
No.reactions	1248:96=13	1152:96=12	1344:96=14	1440:96=15	1248:96=13

Notes

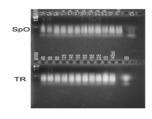
- 1. The stock DNA used for labeling PCR reactions is initially amplified as described in the primary DOP-PCR protocol. This amplified product can then be amplified again as described in the secondary DOP-PCR protocol. It is this secondary amplification product that is then used as the starting material for the SKY labeling PCR reactions.
- 2. Labeling scheme:
 - A-Spectrum Orange
 - **B-Texas Red**
 - C-Biotin
 - D-Spectrum Green
 - E-Digoxygenin

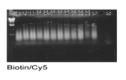
chromosome 1-AE 2-E 3-CDE 4-BC 5-ABDE 6-BE 7-CD 8-D 9-ADE 10-CE 11-A 12-BCDE 13-AD 14-B 15-ABC 16-BCD 17-C 18-ABD 19-AC 20-ACD 21-DE 22-ABCE X-BD Y-ACDE

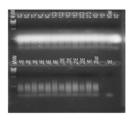
3. PCR program:

Step	Temperature (C)	<u>Minutes</u>
1	94	1
2	56	1
3	72	3 with addition of 1 sec/cycle
4 repeat steps 1-3, 29 time	es	·
5	72	10
6	4	∞

4. The 1% agarose gels migrate to about 500 bp.







R110

Digoxygenin/Cy5.5